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*Full Length Research Paper*

# Some haematological changes in hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal

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**Changes were observed in some haematological parameters of juvenile hybrid catfish *(Heterobranchus longifilis x Clarias gariepinus*) fed raw and 60 min-boiled jackbean seed meal (JBSM) at different dietary levels for 56 days. The haematocrit (packed cell volume, PCV), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) concentration decreased significantly (P<0.05) with increasing dietary JBSM level. Though the mean values of the blood parameters of fish fed diets containing boiled JBSM (PCV = 29.93%; RBC = 1.2 x $10^6$ $mm^{-3}$; WBC = 15.91 x $10^3$ $mm^{-3}$; Hb = 8.31 g/100 ml) showed significant improvement when compared with those fed raw JBSM diets (PCV = 28.68%; RBC = 1.13 x $10^6$ $mm^{-3}$; WBC = 14.63 x $10^3$ $mm^{-3}$; Hb = 8.31 g/100 ml) they were, however, lower and significantly (P<0.05) different from those fed the control diet (PCV = 35.50%; RBC = 1.43 x $10^6$ $mm^{-3}$; WBC = 20.42 x $10^3$ $mm^{-3}$; Hb = 10.62 g/100 ml). However the observed reduction of the blood parameters did not go below the normal range of values recorded for catfish. Further investigation to improve the quality of boiled JBSM is suggested.**

**Key words:** Hybrid catfish, *Heterobranchus longifilis x Clarias gariepinus,* jackbean seed meal, haematology.

## INTRODUCTION

Fish is often the cheapest source of animal protein and is, therefore, important in the diets of the lowest income group (Allison, 2001). After a century of rapid development driven by a combination of technological innovation and increasing demand for fish, global landings of wild-caught fish are thought to be close or exceeding their estimated sustainable level. One of the proposed solutions to the crisis in capture fisheries which contributes eighty percent of total fish production (FAO, 1998) has been resort to fish farming.

In the late 20$^{th}$ century, a “blue revolution” (Coull, 1993) has been witnessed with aquaculture regarded as the world’s fastest growing food production system (Kureshy et al., 2000). However, high quality feed using fishmeal is required to meet the expanding aquaculture production system. The use of wild-caught fish as fishmeal in fish diets puts further pressure on stocks (Naylor et al., 2000). For fish culture to successfully divert efforts away from capture fisheries and to substantially contribute to dietary requirement of low-income groups, efforts have to be directed at substantially replacing the fishmeal component of fish feed.

To meet the protein demand in developing countries where animal protein intake is also grossly inadequate and relatively expensive, intensive research effort is geared towards finding alternative sources of protein from underutilized grain legume seeds (Adaparusi, 1994; Fagbenro, 1999; Osuigwe, 1999). Jackbean (*Canavalia ensiformis*) seed is one of such legumes with a crude protein and amino acid profile that recommends it for use as a substitute for fishmeal in fish feed (Osuigwe, et al.,

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**Table 1.** Chemical composition of jackbean seed meal (g/kg DM).

| Parameter | Raw | Boiled (60 min.) |
|---|---|---|
| Protein (NX6.25) | 282.5 | 254.0 |
| Ether Extract | 29.0 | 28.0 |
| Crude Fibre | 67.3 | 62.1 |
| Ash | 34.4 | 29.2 |
| NFE | 586.8 | 626.7 |
| P (total) | 6.2 | -- |
| Ca | 0.9 | -- |
| Mg | 0.8 | -- |
| Gross energy (Kcal/100g) | 459.32 | -- |

NFE = Nitrogen free extract; P = phosphorus; Mg = magnesium; Ca = calcium.

**Table 2.** Composition of experimental diets.

| Ingredient | Diet No and amount of fishmeal (%) substituted by JBSM | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| | control | 10% | 20% | 40% | 60% | 80% | 100% | 10% | 20% | 40% | 60% | 80% | 100% |
| Fishmeal | 22.0 | 19.80 | 17.60 | 13.20 | 8.80 | 4.40 | 0.00 | 19.80 | 17.60 | 13.20 | 8.80 | 4.40 | 0.00 |
| JBSM* | 0.00 | 4.36 | 8.72 | 17.44 | 26.17 | 38.98 | 43.61 | 4.93 | 9.86 | 19.71 | 29.57 | 39.42 | 49.28 |
| Maize | 35.00 | 32.84 | 30.68 | 26.36 | 22.03 | 15.21 | 12.39 | 32.27 | 29.54 | 24.09 | 17.63 | 12.18 | 6.72 |
| Groundnut meal | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Soyabean meal | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Wheat bran | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Palm oil | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Bone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix ** | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| % Crude Protein | 30.27 | 30.15 | 29.93 | 29.50 | 29.07 | 28.39 | 28.11 | 30.10 | 29.83 | 29.28 | 28.63 | 28.09 | 27.54 |
| ME (Kealkg) *** | 2986 | 2975 | 2964 | 2943 | 2920 | 2903 | 2933 | 2973 | 2916 | 2937 | 2967 | 2943 | 2918 |

* JBSM: diets 2-7 = raw JBSM; 8-13 60 min boled JBSM.
** Vitamin and mineral premix.
*** ME Metabolizable Energy calculated.

2002). It is readily available, cheap and is hardly consumed by man. It however has some antinutritional factors some of which can be reduced to a large extent by processing (Udedibie, 1990). This study was undertaken to establish the effects of feeding raw and boiled JBSM at different dietary levels on some haematological parameters of hybrid catfish (He*terobranchus longifilis x clarias gariepinus*) since fish haematology can be employed to assess fish health (Klinger et al., 1996).

## MATERIALS AND METHODS

Two types of JBSM were obtained by milling the raw seed with hammer mill and subjecting a portion of the milled bean to atmospheric boiling in water (100-105°C) for 60 min. Thereafter, the boiled JBSM was spread out and dried in an oven for 24 h at 60°C. Determinations of the proximate composition of the samples were carried out by AOAC (1990) procedure employing the micro-Kjedahl method for crude protein (CP) and soxhlet method for ether extract (EE). The gross energy of the sample was assayed using adiabatic oxygen bomb calorimetry technique. The milled raw jackbean seed was also subjected to wet digestion with perchloric acid and nitric acid using the Johnson and Ulrich (1959) method. Following digestion, the calcium and magnesium content were determined by atomic absorption spectrophotometer. The phosphorus content was determined on a spectronic 20 spectrophotometry following development of colour with ammonium molybdate. The results were expressed on the basis of dry matter (Table 1). Thirteen practical isonitrogenous (CP 30) and isocaloric (ME 2900 Kca/kg) diets were formulated (Table 2). Diet 1, which served as the control contained no jackbean seed meal but of the same nutritional regime as the other twelve diets. Diets 2, 3, 4, 5, 6, and 7 had the fishmeal component replaced progressively by raw JBSM at 10, 20, 40, 60, 80 and 100%, respectively. In diets, 8, 9, 10, 11, 12, and 13, 60 min boiled JBSM replaced fishmeal at 10, 20, 40, 60, 80 and 100%, respectively. The feedstuffs were then thoroughly mixed and moistened with water. The diets were then molded into small pellets and dried in an oven at 40°C for 24 h and subsequently stored in a freezer until required for use.

The test diets were assigned randomly using CRD to duplicate groups of 20 fish of average total length 18 cm in 20 litre plastic aquaria in static water. The fish were fed once daily for fifty-six days

**Table 3.** Effects of replacement of fishmeal diets with JBSM on the haematocrit (PVC), red blood cell (RBC) count, white blood cell (WBC) count and haemaglobin (Hb) concentration of hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*).

| %Fishmeal substitution | PCV (%) | RBC count ($x10^6mm^{-3}$) | WBC count ($x10^3mm^{-3}$) | Hb concentration (g/100 ml) |
|---|---|---|---|---|
| 0 | $35.43^{fg}$ | $1.43^{hi}$ | $20.69^{g}$ | $10.63^{d}$ |
| 10 | $33.05^{i}$ | $1.38^{j}$ | $18.99^{j}$ | $9.41^{i}$ |
| 20 | $33.04^{i}$ | $1.33^{l}$ | $18.18^{k}$ | $9.21^{k}$ |
| 40 | $31.58^{j}$ | $1.27^{n}$ | $15.91^{l}$ | $8.59^{n}$ |
| 60 | $29.99^{k}$ | $1.17^{o}$ | $15.40^{m}$ | $8.51^{o}$ |
| 80 | $28.91^{l}$ | $1.13^{q}$ | $15.01^{mn}$ | $8.51^{o}$ |
| 100 | $27.58^{m}$ | $1.07^{r}$ | $14.73^{n}$ | $7.98^{q}$ |

Means on the same column with different superscripts are significantly different (P<0.05).

at 3% body weight. Water was replaced every 3 days by siphoning. The water quality parameters were monitored daily and mean values were temperature 28.5 ± 1°C; pH 6.8 ± 0.2; DO 6.4 ± 0.5 mg/l.

Fish were tranquilized with 150 mg/1 solution of tricane methane sulphonate (MS222) (Wagner et al., 1997) for blood collection. Blood samples were collected from 4 fish at the commencement of the feeding trial and bi-weekly subsequently from each aquarium from the caudal artery using 2 ml plastic syringes and needletreated with anti-coagulant and put in sample bottles. Haematocrit (PCV) was determined with microhaematocrit centrifuge by the Wintrobe and Westergreen method as described by Blaxhail and Diasley (1973) with commercially available heparinzed capillary tubes of 25 mm. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined with a haemocytometer with improved Neubauer counting chamber as described by Blaxhall and Diasley (1973). Haemoglobin (Hb) concentration estimates were determined as described by Wedemeyer and Yasutake (1977).

The data obtained were subjected to analysis of variance and differences between means were determined by Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

The mean values for the haematological parameters of juvenile hybrid catfish studied are shown in Table 3. The haematocrit, red blood cell count, white blood cell count and haemoglobin concentration decreased significantly (P<0.05) with increasing dietary JBSM such that fish fed the control diet had the highest values that were significantly (P<0.05) different from the values obtained from fish fed other diets. Variables such as age, sex, dietary state and stress have been known to alter blood values (Barnhart, 1969; McCarthy et al., 1973). Stress factors due to capture, handling and sampling procedures are factors which can cause intra-species haematological variations (Bouck and Ball, 1966). It has also been shown that haemaglobin concentration and haematocrit of fish blood decreases after the stress of capture and transportation (Hattingh and Van Pletzer, 1974). However, bearing in mind that the fish used in this study were siblings kept under laboratory conditions during the period of feeding with test diets and tranquilized with MS222 before handling, the effect of stress resulting from handling must have been minimal. Consequently, only the nutritional state of the fish (given the optimal water quality conditions provided) may have significantly affected the haematological parameters.

Jackbean seed has been shown to contain antinutritional factors (Nakatsu et al., 1996). These include concanavalin-A (Con-A), a lectin (MERCK, 1989), canavanine (Rosenthal, 1992), saponins (Belmar and Morris, 1994a,b.), trypsin and chymotrypsin inhibitors (Ologhobo et al., 1993), polyphenols (Baber et al., 1988), cyanogenic glycosides and terpenoids (Udedibie et al., 1988). Some of these antinutritional factors have been known to adversely affect some haematological parameters. Con-A causes agglutination of red blood cells in monogastrics (Liener, 1979) while saponins are known to cause erythrocyte haemolysis and reduction of blood (Cheeke, 1971). Probably the progressive reduction in the values of the haematological parameters of hybrid catfish was caused by the increasing presence of antinutritional factors with increasing dietary JBSM level. This is in agreement with the findings of Dick et al. (1976) that nutritional toxicity is associated with anaemia. Herman (1970) equally observed that gossypol an antinutritional factor found in some legumes severely reduced blood haematocrit and haemoglobin concentration in rainbow trout.

In terms of the processing type, it was observed that juvenile hybrid catfish fed the control diet had haematocit, red blood cell count, white blood cell count and haemoglobin concentration that were higher and significantly (P<0.05) different from the values of those fed boiled JBSM diets which were in turn higher and significantly different from those fed raw JBSM diets (Table 4). The better performance of fish fed boiled JBSM relative to those fed raw JBSM diets is an indication that boiling significantly improved the quality of some legume seed meals. The improvement may be due to among other factors inactivation of some antinutritional factors present in JBSM as earlier reported by the works of Udedibie and Carlini (1998) and transformation of some

**Table 4.** Effects of replacement of fishmeal in diets with differently processed JBSM on the haematocrit (PVC), red blood cell (RBC) count, white blood cell (WBC) count and haemaglobin (Hb) concentration of hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*).

| Processing type | PCV (%) | RBC count ($x10^6 mm^{-3}$) | WBC count ($x10^3 mm^{-3}$) | Hb concentration (g/100 ml) |
|---|---|---|---|---|
| Raw JBSM | $28.68^g$ | $1.13^i$ | $14.63^h$ | $8.00^e$ |
| 60 min boiled JBSM | $29.93^f$ | $1.20^h$ | $15.91^g$ | $8.31^g$ |
| Control diet | $35.50^d$ | $1.43^e$ | $20.42^d$ | $10.62^c$ |

Means on the same column with different superscripts are significantly different (P<0.05).

of the component nutrients to non-toxic more readily digestible absorbable forms (Rosenthal, 1977). The inferior performance of fish fed boiled JBSM diets relative to those fed the control diet may also be attributed to the effect of heat treatment which renders JBSM protein deficient and unbalanced by destroying some amino acids. Bressani et al. (1987) reported that heat treatment not only reduced the level of lysine but also destroyed methionine (both essential amino acids) in jackbean, thus reducing the biological value of JBSM protein. Viola et al. (1983) equally reported that heat treatment causes deficiency and inbalance in the protein quality of legumes. The observed reduction in haematological parameters in hybrid catfish fed boiled JBSM diets in this study therefore conforms with the report of Tacon (1992) that nutritionally deficient diets cause decrease in haemaglobin concentration, reduced haematocrit and red blood cell count. Physiologically, haemoglobin is crucial to the survival of fish being directly related to the oxygen binding capacity of blood. However, the reduction observed in this study (between 7.98-10.63 g/100 ml) may not have had a deleterious effect on hybrid catfish, given that the values are within the normal range recorded for African catfish (Erondu et al., 1993; Musa and Omoregie, 1999). So also were the values obtained for haemotocrit (between 27.58 – 35.50%), red blood cell count (between 1.07-1.43 x $10^6$ $mm^{-3}$) and white blood cell count (between 14.63-20.69 x $10^3$ $mm^{-3}$). The haematocrit values also fall within the normal range of 20-35% and rarely greater than 50% for fish (Clark et al., 1979).

This study therefore highlights the fact that though hybrid catfish could be fed diets containing JBSM for 56 days without apparent deleterious effects on the fish.Also, boiling JBSM before use to a large extent improved the quality of the diet as regards the blood profile. Further investigation to study the effect of incorporating supplementary amino acid observed to be deficient in boiled JBSM on the haematological parameters is advocated.

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